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Mitochondrial DNA analysis and zoogeography of two species of silky desert mice, *Eligmodontia*, in Patagonia

By Jeanna R. Hillyard, C. J. Phillips, E. C. Birney, J. A. Monjeau, and R. S. Sikes

Department of Biological Sciences, Illinois State University, Normal, Illinois, USA, James Ford Bell Museum of Natural History and Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, Minnesota, USA, and Departamento de Ecología, Universidad Nacional del Comahue, Bariloche, Río Negro, Argentina

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Abstract

Historically, silky desert mice of the genus *Eligmodontia* have presented mammalogists with a complex and somewhat vexing taxonomic problem. At various times silky desert mice have been assigned to several species or to only one or two species. Most recently karyological evidence has suggested the presence of at least two morphologically cryptic species, *E. morgani* and *E. typus* in Patagonia. To further elucidate this issue, we used a combination of karyological data and DNA sequences from the mitochondrial cytochrome b protein-coding gene to test the hypothesis that there are at least two genetically distinguishable and reproductively isolated species in Patagonia. By this means we showed that *E. morgani* and *E. typus* can be recognized readily by their karyotypes and mtDNA. In fact they exhibit more than 10% divergence in a 348-base pair region of cytochrome b. Within each species the mtDNA sequences enabled us to identify numerous maternal lineages. Results of PAUP analyses used to place these lineages in a geographic context suggested that the oldest lineage in the sample of each species was rare and occurred in the northwestern portion of the study area. Each species also was characterized by a common, geographically widespread "star" lineage. Typically, at each locality we found the star lineage and one or more local, related lineages. These data are consistent with a historical bottleneck, rapid expansion through the star lineage, and subsequent settling in and production of new lineages. The unequivocal identification of individuals based on mtDNA, not previously possible on the basis of morphology alone, allowed us to begin mapping distribution of these two largely allopatric species. The data suggest that *E. morgani* occurs in more mesic habitats, whereas *E. typus* occupies more arid areas mostly to the east of the range of *E. morgani*.

Introduction

Silky desert mice of the genus *Eligmodontia* are small phyllotine rodents that occur over a large geographic region encompassing western Bolivia, southern Peru, northern Chile, and Argentina. In general terms these mice are thought to inhabit arid scrubland habitats characterized by as little as 150–500 mm annual precipitation and at least some of the species are capable of using halophytic plants as a source of water (Mares 1977). However, detailed zoogeographic, ecological, and physiological studies of silky desert mice have been hampered by uncertainty as to species identifications and changing taxonomic arrangements. Presently, the ranges of putative species and their habitat requirements essentially are unknown.

The silky desert mice have had a complicated taxonomic history that dates back to early work by Phillipi (1896), Thomas (1916), and Allen (1901). More recently, Hershko-

VITZ (1962) lumped them into a single species (*E. typus*, as shown in REDFORD and EISENBERG 1992), whereas MUSSER and CARLETON (1993) recognized four species. For the most part, the confusion about *Eligmodontia* taxonomy can be traced to the facts that (a) sample sizes have been small and from widely scattered geographic locations and (b) traditional morphological features have failed to consistently delineate species (SIKES et al. 1997). The difficulties of working with *Eligmodontia* are especially evident in the Patagonian region of Argentina. In this region one could argue on morphological grounds (as HERSHKOVITZ did in 1962) that a single species occurs from the Andean foothills on the west to the arid Atlantic coast on the east. On the other hand, karyotypic analyses (including G- and C-banding) of specimens collected in various parts of Patagonia have revealed two distinctive cytotypes, one with a 2N= 43–44 karyotype and the other with 2N = 32–33. Based on geography, ORTELLS et al. (1989) concluded that the 2N = 43–44 karyotype was associated with *E. typus* and KELT et al. (1991) associated the 2N = 32–33 karyotype with *E. morgani* based on the fact the specimens with this arrangement were captured within 70 km of the likely type locality of this species (ALLEN 1901).

In contrast to morphological evidence and some taxonomic arrangements, the karyotypic evidence thus clearly indicates the existence of at least two reproductively isolated species of *Eligmodontia* in Patagonia. However, karyotypic data are not available for most museum specimens of *Eligmodontia* and research collections are incomplete in terms of geographic and ecological representation. Thus, in our overall investigation we sought to further elucidate the genetics of the two cytotypes, to expand the geographic and ecological representation of specimens that could be assigned reliably to reproductively isolated units labeled as *E. morgani* or *E. typus*, and use these animals to test more fully their morphological characteristics. In the present study we use mtDNA sequences and karyotypic data to evaluate genetic divergence between and within these species and to develop hypotheses concerning historical biogeography. These data provide new information on the distribution of the two species and document areas of sympatry. A second contribution (SIKES et al. 1997) contrasts morphological divergence with the patterns of karyotypic and genetic divergence presented herein.

Methods

Sixty-seven specimens of *Eligmodontia* were used in the present analyses. Voucher specimens of 66 of the mice were deposited in the collection of the James Ford Bell Museum of Natural History, University of Minnesota, St. Paul (MMNH specimen numbers). One animal (FMNH 133049) used in the study came from the Field Museum of Natural History, Chicago: it served as a cytotypic voucher from KELT et al. (1991) and represented *E. morgani*.

Mice were captured at 16 localities in Río Negro, Chubut, and Santa Cruz provinces (41° to 44° south latitude; 63° to 71° west longitude). Liver, kidney, and heart tissues were taken in the field and quick frozen and stored in liquid nitrogen. Chromosome spreads also were prepared in the field for selected specimens following the methods of PATTON (1967) as modified by LEE and ELDER (1980).

To obtain genetical data on Patagonian *Eligmodontia*, we elected to use DNA sequencing of a 348-base pair (bp) region of cytochrome b, which is a mitochondrial protein-coding gene. The tempo and mode of evolution differs among types of mitochondrial genes (PUMO et al. 1992), but cytochrome b is known to provide good resolution for inter- and even intra-specific geographic analyses of rodents and other kinds of mammals (e. g., SMITH and PATTON 1991; IRWIN et al. 1991). Given the rate of evolution in the cytochrome b gene (estimated at 2–4%/1×10^6 years, BROWN et al. 1979; MARTIN et al. 1992), we anticipated that reproductively isolated rodent species should exhibit differences in cytochrome b DNA sequences. Mitochondrial DNA (mtDNA) offers several advantages including the opportunity to compare the data set to those from other rodents and, possibly, to trace the zoogeographic history of species in a geographic region (AVISE 1994).

In the laboratory, total DNA was prepared from tissues by the proteinase K method (KOCHER et al. 1989). After extraction by phenol-chloroform-isoamyl alcohol, samples were subjected to the polymer-

ase chain reaction (PCR) using primers MVZ04 and MVZ05 designed for a region of the protein-coding cytochrome b gene in the mitochondrial genome (Smith and Patton 1991). Amplification (Saiki et al. 1985, 1988) was performed with Taq polymerase (Perkin-Elmer) for 30 cycles. Excess primer and nucleotides were removed from PCR products by using a GENECLEAN II kit (BIO 101) and following manufacturer's directions. Purified, amplified mtDNA was sequenced using Sequenase Version 2.0 (United States Biochemical) and [^{35}S]dATP or by means of an ABI-310 automatic sequencing system (Perkin-Elmer). Finally, DNA sequence alignment was performed with the IBI MacVector (version 4.1) software and phylogenetic analyses were done with PAUP v 3.0 (Swofford 1993). The mtDNA sequences have been submitted to GenBank.

Results

Our first step was to compare mtDNA sequence data from 15 specimens for which we also had karyotypic data. Our reference point was a mouse (2N = 32–33 cytotype) that previously had been assigned to *Eligmodontia morgani* on the basis of geographic origin (Kelt et al. 1991; FMNH 133049, Tab. 1). Mitochondrial DNA from this mouse was se-

Table 1. Collection localities and mtDNA lineages for the voucher specimens of *Eligmodontia* used in the combined DNA sequence and karyological analyses. Abbreviations: FMNH (Field Museum of Natural History number; specimen from study by Kelt et al. 1991); MMNH (James Ford Bell Museum of Natural History number).

Specimen number	Assigned mtDNA haplotype lineage	Species	Locality	
Karyotype: 2N = 32–33: mtDNA Haplotype M				
FMNH 133049	M1	*E. morgani*	Ea. La Vizcaina	46°55′S, 70°50′W
MMNH 17097	M12	*E. morgani*	Tembrao	41°08.5′S, 66°18.5′W
MMNH 17112	M8	*E. morgani*	Chile Chico	46°33′S, 70°56′W
MMNH 17287	M3	*E. morgani*	Ea. El Rincón	46°59.8′S, 70°42.7′W
MMNH 17356	M3	*E. morgani*	La Subida	43°58.55′S, 70°22.97W
MMNH 17321	M10	*E. morgani*	Ea. La Escondida	45°19.4′S, 69°50.1′W
MMNH 17322	M9	*E. morgani*	Ea. La Escondida	45°19.4′S, 69°50.1′W
Karyotype: 2N = 43–44: mtDNA Haplotype T				
MMNH 17052	T18	*E. typus*	Viedma	40°56.4′S, 63°01.3′W
MMNH 17053	T6	*E. typus*	Viedma	40°56.4′S, 63°01.3′W
MMNH 17054	T15	*E. typus*	Viedma	40°56.4′S, 63°01.3′W
MMNH 17055	T17	*E. typus*	Viedma	40°56.4′S, 63°01.3′W
MMNH 17056	T1	*E. typus*	Viedma	40°56.4′S, 63°01.3′W
MMNH 17087	T6	*E. typus*	Aguada Cecilio	40°51.49′S, 65°48.35′W
MMNH 17172	T14	*E. typus*	El Pedrero	46°48.1′S, 69°37.6′W
MMNH 17173	T19	*E. typus*	El Pedrero	46°48.1′S, 69°37.6′W

quenced and the cytochrome b sequence was labeled as the M haplotype. We next sequenced the remaining 14 animals. Six of these had karyotypes consistent in number and morphology with the specimen from the study by KELT et al. (1991). Although none had a cytochrome b sequence identical to the M haplotype, there were five new sequences (one shared by two mice) that differed from the M haplotype by only 1–6 nucleotide bases. Thus, these six animals (including two that had been obtained within 40 km of the type locality of *E. morgani*) were identified as *E. morgani* and the five mtDNA sequences (one shared, Tab. 1) were labeled numerically as "lineages" of the M haplotype (M12, M8, M3, M10, M9). The reference animal (FMNH 133049) was labeled M1. Eight animals were assigned to *E. typus* on the basis of having karyotypes consistent with that described for the species (2N = 43–44) by ORTELLS et al. (1989). The mtDNA cytochrome b sequences from these animals differed markedly from all six lineages in the *E. morgani* M haplotype. Indeed, among the animals assigned to *E. typus* on basis of karyotype, their mtDNA sequences typically differed from the M haplotype lineages by more than 34 nucleotide bases (>10% sequence difference) and were designated as representing the T haplotype. Among the specimens of *E. typus*, there were seven different mtDNA sequences differing by 1–6 nucleotide bases. These were labeled numerically as T1, T6, T14, T15, T17, T18, and T19 (Tab. 1). Collectively, the lineages of the M and T haplotypes from animals of known karyotype were analyzed by means of PAUP and the two groups of mtDNA lineages formed two clades in complete congruence with the chromosomal data (Fig. 1).

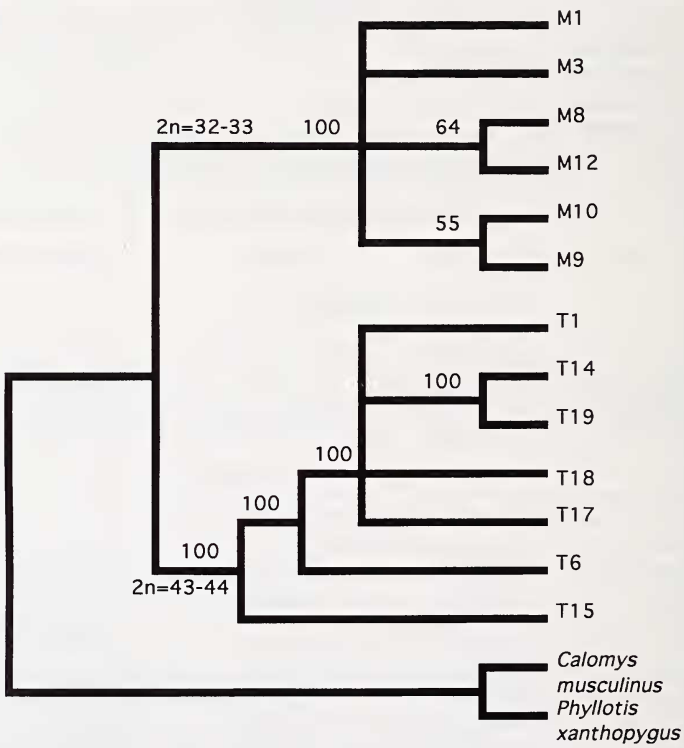


Fig. 1. Fifty percent majority rule consensus PAUP tree with analysis (100 iterations) of the 348-bp region of cytochrome b of two species of *Eligmodontia*. Bootstrap values are shown above each branch. The diploid chromosomal number is listed for each clade. Homologous DNA sequences from two genera of Patagonian rodents, *Phyllotis xanthopygus* and *Calomys musculinus*, were used as outgroups. The tree length is 152 steps, the consistency index is 0.836, and the retention index is 0.896.

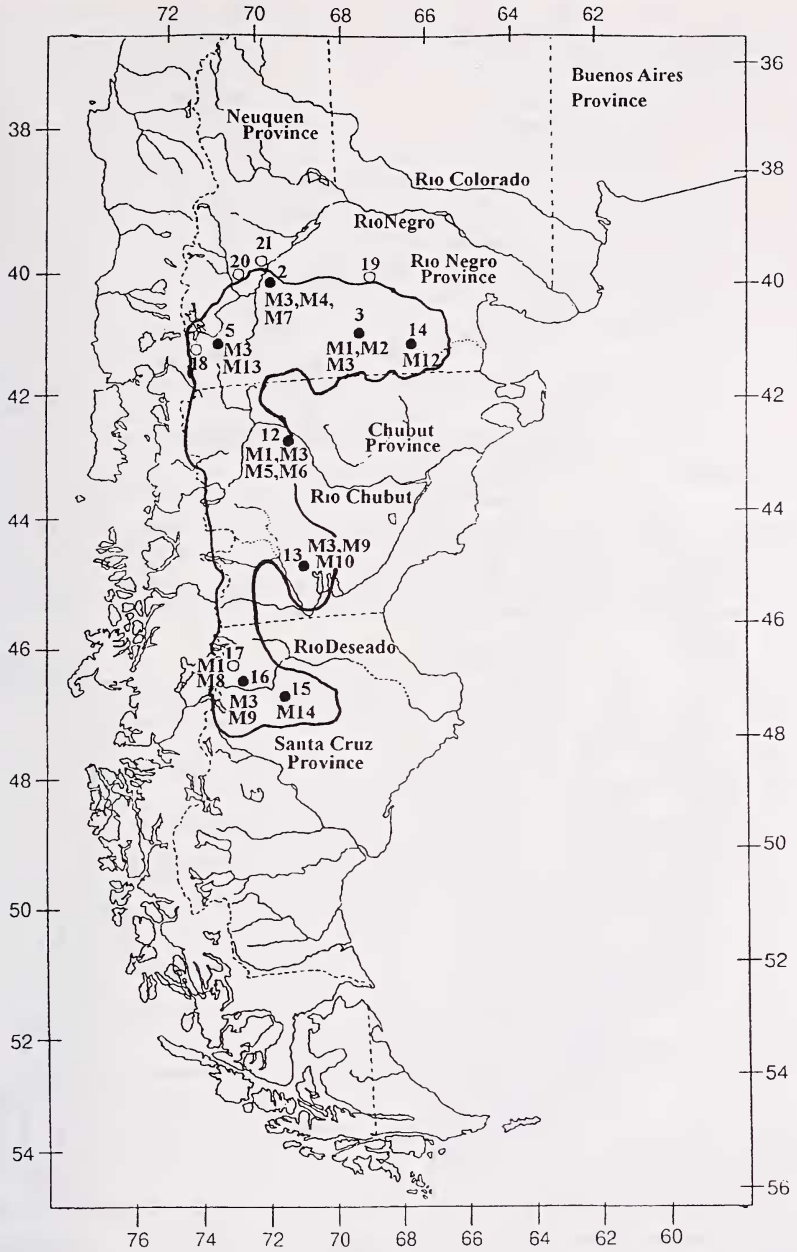


Fig. 2. Localities of *Eligmodontia morgani* in Río Negro, Chubut, and Santa Cruz provinces of Argentina (confirmed by chromosomal, mtDNA data, or both). Open circles indicate localities from Ortells et al. (1989) and Kelt et al. (1991). The closed circles are localities for specimens examined in the present study. MtDNA haplotypes are listed next to collection localities. The solid line shows an area known as the Extra-Andean Occidental Megabiozone (del Valle et al. 1995).

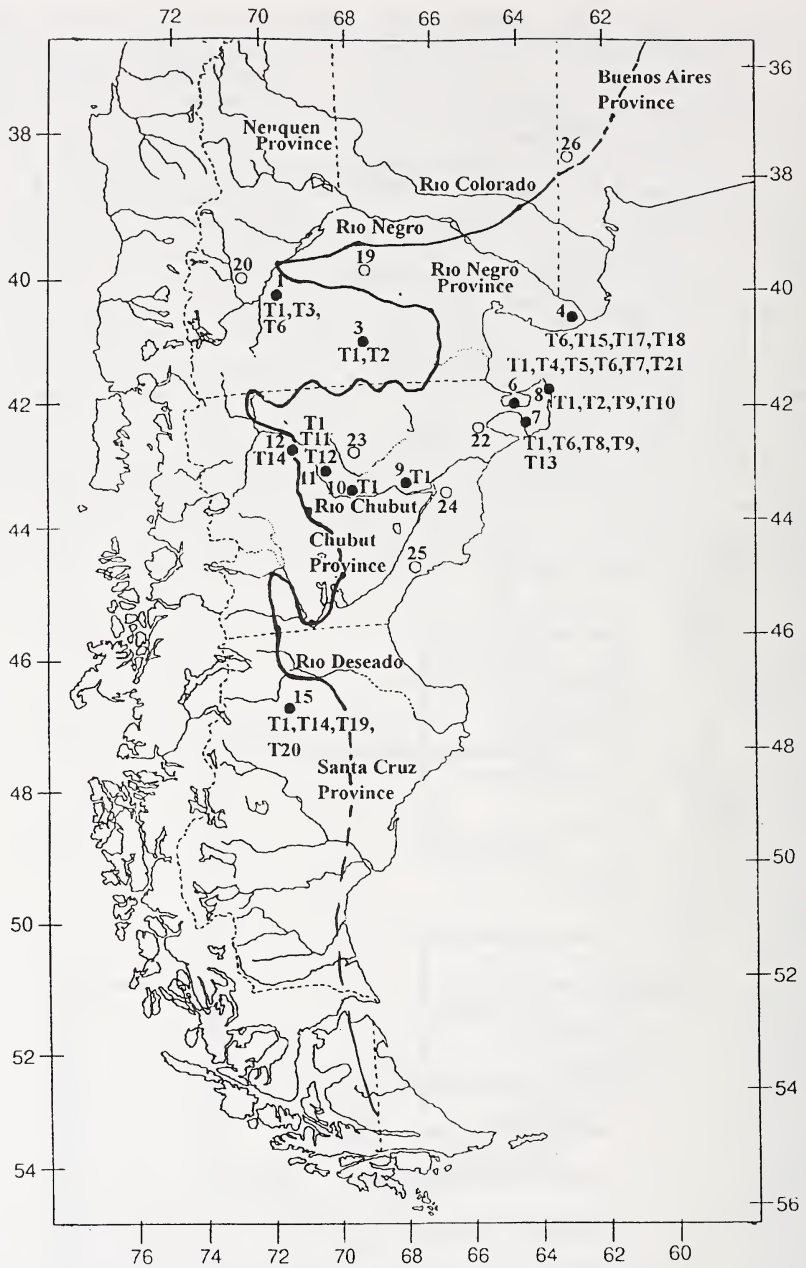
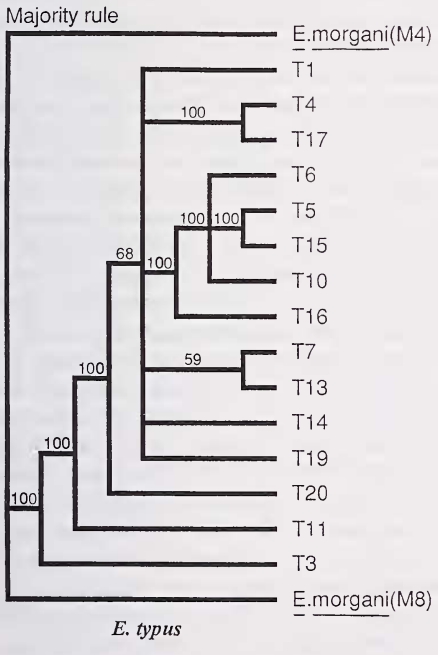


Fig. 3. Localities of *Eligmodontia typus* in Buenos Aires, Neuquen, Río Negro, Chubut, and Santa Cruz provinces of Argentina (confirmed by chromosomal, mtDNA data, or both). Open circles indicate localities of specimens from ORTELLS et al. (1989), KELT et al. (1991), and ZAMBELLI et al. (1992). The closed circles are localities for specimens examined in the present study. MtDNA lineages are listed next to collection localities. The solid line coincides with an area known as the Extra-Andean Oriental Megabiozone (DEL VALLE et al. 1995). The dashed lines estimate the continuation of this vegetation type and our distributional hypothesis for *E. typus* outside the boundaries of this study area.

The second part of our investigation involved sequencing the same region of the cytochrome b gene in the remaining 52 "unknown" specimens (i.e., no karyotypic data) and using a PAUP analysis to assign each animal to a species based on its mtDNA. As a result, we had a total sample of 36 specimens of *E. typus* from 11 localities. Within the total sample for this species, there were T21 haplotype lineages that differed by as many as 9 bases (2.6%). The total sample of *E. morgani* was 28 animals from 10 localities. In this sample there were 14 M haplotype lineages that diverged by as much as 2.3%.

In terms of the molecular evolution of the cytochrome b gene we found a striking difference between the two species. In *Eligmodontia typus* we found 38 variable positions in the T haplotype: 79% of these were third position C–T transitions; 15.8% were third position A–G transitions; and the remaining two were first and third position transversions, giving a transition : transversion ratio of 14 : 1. Although the total divergence within the *E. morgani* M haplotype was similar to that in the T haplotype, the pattern of molecular evolution was different. In the M haplotype we found 23 variable positions: 43% of the substitutions were third position C–T transitions; 21.7% were third position A–G transitions; and the remaining substitutions either were first or second position transitions or were transversions (the latter being 21.7% of the total number of substitutions, giving a transition : transversion ratio of only 4 : 1).

For a phylogeographic and historical perspective based on mtDNA sequences we undertook PAUP analyses for each species (Figs. 4, 5). In each case we used two of the mtDNA lineages from one species to polarize the lineage tree for the other species. No strong evidence of geographic structuring was evident in either species. That is, no clades



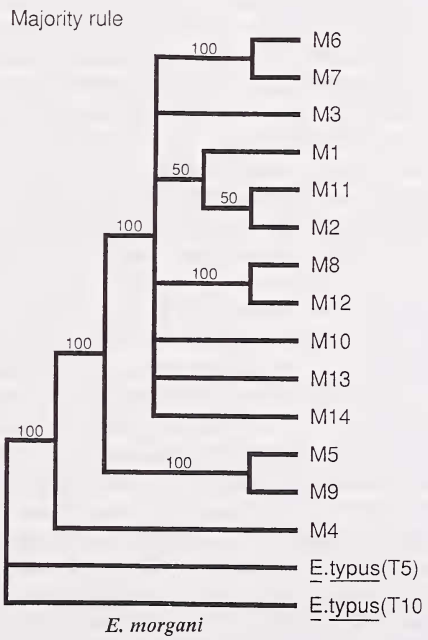


Fig. 4. Majority rule consensus PAUP tree with bootstrap analysis (100 iterations) of 15 mtDNA haplotypes of *Eligmodontia typus* using sequences from *Eligmodontia morgani* as outgroups. Bootstrap values are shown above each branch.

Fig. 5. Majority rule consensus PAUP tree with bootstrap analysis (100 iterations) of 14 mtDNA haplotypes of *Eligmodontia morgani* using sequences from *Eligmodontia typus* as outgroups. Bootstrap values are shown above each branch.

of lineages in either species could be identified as "characteristic" of a specific geographic area (Figs. 4, 5). In each species one particular lineage predominated in terms of numbers of animals and geographic distribution: in *E. morgani* 12 mice (43%) represented the M3 maternal lineage, which was found at six localities as much as 700 km apart; in *E. typus*, 10 mice (28%) represented the T1 lineage, which was obtained all but one locality where the species was found (Fig. 2). Polarization of the PAUP tree enabled us to hypothesize the basal lineage(s) among all of the M and T haplotype lineages. In *E. morgani*, the M4 lineage, obtained at a locality near Mencué in northwestern Río Negro Province, was hypothesized as the oldest extant lineage in our sample (Fig. 4). In *E. typus*, the T3 lineage from Ea. María Sofía in northwestern Río Negro Province (< 70 km from the Mencué locality) was hypothesized by the PAUP analysis as the extant basal lineage in the sample (Fig. 4). In both species these basal lineages were rare (isolated from a single animal).

Discussion

Although silky desert mice of the genus *Eligmodontia* in Patagonia show little morphological divergence (HERSHKOVITZ 1962; SIKES et al. 1997), recent karyological studies by ORTELLS et al. (1989), KELT et al. (1991), and ZAMBELLI et al. (1992) clearly established the existence of at least two karyological cytotypes in the region. Moreover, because it is highly improbable that animals with 2N = 32–33 and 2N = 43–44 chromosomal arrangements could interbreed successfully (see also KELT et al. 1991), it also can be concluded that the cytotypes are reproductively isolated. Our mtDNA data are fully congruent with the chromosomal data and the absence of shared haplotypes, or lineages, between cytotypes supports the logical conclusion of reproductive isolation between *E. morgani* and *E. typus*.

Collectively, the karyological and mtDNA data can be used to shed some light on the likely history of *E. morgani* and *E. typus*. Insofar as chromosomal divergence is concerned, the difference between these two species presently appears to be a combination of small Robertsonian rearrangements and an array of pericentric inversions, tandem translocations, and, probably, euchromatic amplifications and deletions (ORTELLS et al. 1989). From this one might infer that chromosomal differences accumulated over time, perhaps after the parent metapopulation had been physically subdivided. Alternatively, there might have been an initial event that resulted in reproductive isolation or limited fertility between cytotypes within a population. In terms of the mtDNA data, the difference between the M and T haplotypes (> 10%) seems to suggest a relatively old divergence. Presently there is no way to calibrate the rate of molecular evolution of cytochrome b in *Eligmodontia*, but an application of generalized rate in mammals (BROWN et al. 1979; MARTIN et al. 1992) would imply a coalescence of the two haplotypes in the early Pleistocene or late Pliocene. More importantly, the divergence between the M and T haplotypes is far greater than the divergence within each (> 10% vs < 2.6%) and, thus, the historical starting point for the two haplotypes considerably predates the origins of any of the known extant lineages. A deep history of divergence could be indicative of an early, rapidly occurring physical split in the parent population (from geographic or chromosomal causes) as opposed to a speciation process that was (a) recent, (b) gradual, or (c) characterized by periodic hybridization (LEHMAN et al. 1991; HUGHES and CARR 1993). Finally, it should be noted that the foregoing interpretation is not reflected in the morphology of these mice. The striking physical similarity between the two species, which caused the original taxonomic complications, belies their dramatic genetic and karyotypic difference. This similarity raises additional questions about the history of the species and the selection pressures they have experienced (SIKES et al. 1997).

The mtDNA lineages in our samples of both *E. morgani* and *E. typus* that were hypothesized as being the oldest were found in northwestern Patagonia. In both species

these old lineages appear to be rare (isolated from single animals), but this is what one might expect because of stochastic lineage extinction (Moritz 1994; Avise 1996). The geographic positioning of old lineages could be misleading because other, older, lineages might be uncovered by additional sampling. However, it also is possible that our data are indicative of the geographic source of the modern population of each species (Avise 1996). For example, the hypothesized oldest lineage in our sample of *E. morgani* (M4) was found in northwestern Río Negro Province rather than in the southern or eastern part of the present range (Fig. 2). From this information we could hypothesize that the modern Patagonian population of *E. morgani* originated somewhere in the steppe-like habitats east of the Andes in northwestern Patagonia (c. f. the Extra-Andean Occidental biozone of del Valle et al. 1995). The data for *E. typus* are interesting because although the species presently is abundant along the Atlantic coast, the hypothesized basal lineage (T3) was found in northwestern Patagonia. So, although we had anticipated that the *E. typus* population might be traced to the Atlantic coastal region north of Patagonia, the mtDNA data seem to imply that the modern Patagonian population of *E. typus* was derived from the west rather than the coastal region. This interpretation would suggest that modern populations of both *E. typus* and *E. morgani* trace to the same general geographic region. Although it is possible that both survived the end of the Pleistocene in a refugium in the eastern shadow of the Andes, this conclusion is limited by the geographic scope of our study. For example, *E. typus* also occurs well to the northeast of our region and we presently have no mtDNA data from there. Thus, the potential hypothesis that the two species shared a refugial zone is speculative until additional specimens are collected both north and south of our present study area.

It also should be noted that our data set is unusual in that the hypothesized basal lineages in both species were found in only a single locality and represented by a single individual in our sample. Sometimes, basal lineages are the most common and geographically widespread within a species (Crandall and Templeton 1993) but a pattern similar to the one seen in *Eligmodontia* also has been observed in the Jamaican fruit bat, *Artibeus jamaicensis*. In this instance a derived lineage occurs from the Yucatán Peninsula of México through the Caribbean, whereas basal lineages are found only on the mainland or individual islands (Phillips et al. 1991). Additionally, it should be noted that our data overall are similar to phylogeographic mtDNA data from other species of vertebrates (Avise 1987) and this might reflect a common post-Pleistocene phenomenon of rapid range expansion.

Beyond the geographic polarity described above, the PAUP analyses did not reveal any geographic structuring in the distribution of the mtDNA lineages. Thus, within the limits of genetic resolution provided by the cytochrome b sequences there is no indication that animals in some portion of the studied species ranges have been isolated for long periods of time. In fact, in each species there is a particular lineage (T1 and M3) represented at virtually every locality sampled (Figs. 2, 3). The pattern of one common, widespread, lineage with numerous associated local lineages that could be derived from it by a small number of nucleotide base substitutions, as observed in both species in our study, has been referred to as a star lineage. Our tentative interpretation is that both *E. typus* and *E. morgani* experienced population bottlenecks and then underwent population expansions and spread fairly quickly into their current ranges.

It is reasonable to imagine these bottlenecks occurring in northwestern Patagonia where we found the hypothesized oldest lineage of each because this is a region where the Oriental (occupied primarily by *E. typus*) and Occidental (occupied primarily by *E. morgani*) biozones interdigitate with one another depending on elevation (Monjeau et al. in press). During glacial retreats and advances of the Pleistocene (Clapperton 1993) there would have been alternating episodic expansions and contractions of these biozones caused by climatic changes and these would have resulted in the development of small

isolated patches of first one and then the other habitat type, and hence small populations of first one and then the other species of *Eligmodontia*. Such small populations are the bottlenecks we envisage. The present day legacy of this history may be greater for *E. morgani* than for *E. typus* because the latter species has a much broader overall distribution. Although this interpretation of our data is consistent with the landscape history of this region, other interpretations are possible and ours requires further testing.

The genetic delineation of these two species of silky desert mice further our understanding of their respective distributions in Patagonia. Our data, based on specimens for which unequivocal identifications are available, largely corroborate the conclusions of KELT et al. (1991) that *E. morgani* is more restricted to western Patagonia, whereas *E. typus* occurs broadly throughout the central and eastern portions of Patagonia (Figs. 2, 3). However, these data do extend the known distributions of *E. morgani* further eastward than previously known. The distributions of these species in Patagonia appear to follow the Megabiozones described for this area by DEL VALLE et al. (1995) with *E. typus* occurring primarily in the Extra-Andean Oriental biozone and *E. morgani* primarily in the Extra-Andean Occidental biozone (MONJEAU et al. in press). At most localities we captured one species or the other, but we caught both species within walking distance of our camps at three ecotonal localities – Meseta de Somuncura, Ea. Mallín Blanco north of Pampa de Agnia, and Meseta El Pedrero. These data corroborate the earlier report by ZAMBELLI et al. (1992) that these morphologically cryptic species sometimes are sympatric.

The data presented herein document substantial genetic divergence between *E. morgani* and *E. typus* that is in sharp contrast to their high degree of morphological similarity. The patterns of intraspecific genetic divergence pose questions concerning patterns of gene flow and lineage divergence on a local scale, but the deep divergence between the T and M haplotypes raises questions concerning their respective biogeographic histories.

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Zusammenfassung

Analyse der Mitochondrien-DNA und Zoogeographie von zwei Arten von Wüstenseidenmäusen, Eligmodontia, in Patagonien

Wüstenseidenmäuse der Gattung *Eligmodontia* haben den Säugetierkundlern seit langem ein komplexes und etwas ärgerliches taxonomisches Problem bereitet. Zu verschiedenen Zeiten wurden Wüstenseidenmäuse als zu einer oder zu mehreren Arten gehörig betrachtet. In jüngster Zeit haben karyologische Befunde das Vorhandensein von mindestens zwei morphologisch kryptischen Spezies nahegelegt. Um die Hypothese zu prüfen, daß in Patagonien mindestens zwei genetisch unterscheidbare und reproduktiv isolierte Arten leben, haben wir in der vorliegenden Arbeit eine Kombination

aus karyologischen Daten und DNA-Sequenzen des mitochondrialen Cytochrom-b-Gens herangezogen. Mit diesen Methoden konnten wir zeigen, daß *E. morgani* und *E. typus* anhand ihres Karyotyps und ihrer mtDNA leicht voneinander unterschieden werden können. So zeigten sie mehr als 10% Sequenzdivergenz in einem 348bp langen Abschnitt des Cytochrom-b-Gens. Innerhalb jeder Art konnten zahlreiche maternale Linien identifiziert werden. Die Ergebnisse von PAUP-Analysen, die hinsichtlich des Zusammenhanges der Linien mit der geographischen Verbreitung der untersuchten Tiere angestellt wurden zeigten, daß bei jeder Art die älteste Linie selten und auf den nordwestlichen Teil des Untersuchungsgebietes beschränkt war. Jede Art war auch durch eine häufige, geographisch weitverbreitete „Hauptlinie" gekennzeichnet. An jedem Sammelort wurde typischerweise die Hauptlinie, nebst einer oder mehrerer nahe verwandter lokaler Linien gefunden. Diese Daten stimmen mit der Annahme eines historischen genetischen Engpasses, der raschen Ausbreitung der überlebenden Hauptlinie und der anschließenden Weiterverbreitung unter Herausbildung lokaler Linien überein. Die eindeutige Identifikation von Individuen auf der Basis mitochondrialer DNA, die bisher mittels ausschließlich morphologischer Daten nicht möglich war, erlaubte uns den Beginn der Kartierung der Verbreitung der beiden weitgehend allopatrischen Arten. Nach diesen Daten kommt *E. morgani* in eher gemäßigten Habitaten, *E. typus* in eher ariden Gebieten, östlich des Verbreitungsgebietes von *E. morgani*, vor.

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Authors' addresses: JEANNA R. HILLYARD and C. J. PHILLIPS, Department of Biological Sciences, Illinois State University, Normal, IL 61790-4120, USA; E. C. BIRNEY and R. S. SIKES, James Ford Bell Museum of Natural History and Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN 55108 USA; J. A. MONJEAU, Departamento de Ecología, Universidad Nacional del Comahue, CC. 1336, Bariloche, Argentina